MODIFIED FGF-21 POLYPEPTIDES AND THEIR USES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 15/953,091 filed Apr. 13, 2018, which is a divisional of U.S. application Ser. No. 15/292,700, filed Oct. 13, 2016, now U.S. Pat. No. 9,975,936, which is a divisional of U.S. patent application Ser. No. 14/680,543, filed Apr. 7, 2015, now U.S. Pat. No. 9,517,273, which is a divisional of U.S. patent application Ser. No. 13/732,522, filed Jan. 2, 2013, now U.S. Pat. No. 9,079,971, which is a divisional of U.S. patent application Ser. No. 13/051,953, filed Mar. 18, 2011, now U.S. Pat. No. 8,383,365, which is a divisional of U.S. patent application Ser. No. 12/051,830, filed Mar. 19, 2008, now U.S. Pat. No. 8,012,931, which claims the benefit of U.S. Provisional Application No. 60/988,060, filed Nov. 14, 2007, and also claims the benefit of U.S. Provisional Application No. 60/921,297, filed Mar. 30, 2007, the disclosures of all of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

This application includes a sequence listing which has been submitted via EFS-Web in a file named "43270o1009.txt" created on Jun. 17, 2019 and having a size of 87,097 bytes, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to FGF-21 polypeptides optionally modified with at least one non-naturally-encoded amino acid.

BACKGROUND OF THE INVENTION

Fibroblast growth factors are large polypeptides widely expressed in developing and adult tissues (Baird et al., Cancer Cells, 3:239-243, 1991) and play crucial roles in multiple physiological functions including angiogenesis, mitogenesis, pattern formation, cellular differentiation, metabolic regulation and repair of tissue injury (McKeehan et al., Prog. Nucleic Acid Res. Mol. Biol. 59:135-176, 1998; Burgess, W. H. et al., Annu. Rev. Biochem. 58:575-606 (1989). The prototypic fibroblast growth factors (FGFs), FGF-1 and FGF-2, were originally isolated from brain and pituitary as mitogens for fibroblasts. FGF-3 was identified to be a common target for activation by the mouse mammary tumor virus (Dickson et al., Ann. N.Y. Acad. Sci. 638:18-26 (1991); FGF-4 to FGF-6 were identified as oncogene products (Yoshida et al., Ann. NY Acad. Sci. 638:27-37 (1991); Goldfarb et al., Ann. NY Acad. Sci 638:38-52 (1991); Coulier et al., Ann. NY Acad. Sci. 638:53-61 (1991)). FGF-10 was identified from rat lung by homology-based polymerase chain reaction (PCR) (Yamasaki et al., J. Biol. Chem. 271:15918-15921 (1996)). FGF-11 to FGF-14 (FGF homologous factors (FHFs) 1 to 4) were identified from human retina by a combination of random cDNA sequencing, database searches and homology-based PCR (Smallwood et al., Proc. Natl. Acad. Sci. USA 93:9850-9857 (1996)). FGF-15 was identified as a downstream target of a chimeric homeodomain oncoprotein (McWhirter et al., Development 124:3221-3232 (1997)). FGF-16, FGF-17, and FGF-18 were identified from rat heart and embryos by homology-based PCR, respectively (Miyake et al., Biochem. Biophys. Res. Commun. 243:148-152 (1998); Hoshikawa et al. Biochem. Biophys. Res. Commun. 244: 187-191 (1998); Ohbayashi et al., J. Biol. Chem. 273:18161-18164 (1998)). FGF-19 was identified from human fetal brain by database search (Nishimura et al., Biochim. Biophys. Acta 1444:148-151 (1999)). They have a conserved ~120-amino acid residue core with ~30 to 60% amino acid identity.

Animal models, overexpression, and analysis of naturally occurring mutations implicate fibroblast growth factors and their receptors in a wide range of diseases (e.g. Wilkie et al., Current Biology, (1995) 5:500-507; Pugh-Humphreys et al, In: The Cytokine Handbook, A. Thomson ed, 2nd edition, Academic Press, Harcourt Brace & co. publishers, London, pp 525-566) suggesting that regulation of activity could be used for treatment. For example, inhibition of fibroblast growth factor-2 by the compound Suramin prevents neovascularisation and tumor growth in mice (Pesenti et al., British Journal of Cancer, 66:367-372). Fibroblast growth factors also function in angiogenesis (Lyons, M. K., et al., Brain Res. (1991) 558:315-320), wound healing (Uhl, E., et al., Br. J. Surg. (1993) 80:977-980, 1993), astrogliosis, glial cell proliferation and differentiation (Biagini, G. et al., Neurochem. Int. (1994) 25:17-24), cerebral vasodilation (Tanaka, R. et al., Stroke (1995) 26:2154-2159), and neurotrophic/neuromodulatory processes.

Fibroblast growth factor also has multiple positive effects including blood flow and protection from calcium toxicity to improve outcome in cerebral ischemia (Mattson, M. P. et al., Semin. Neurosci. (1993) 5:295-307; Doetrocj. W. D. et al., J. Neurotrauma (1996) 13:309-316). Basic FGF treatment promotes neoangiogenesis in ischemic myocardium (Schumacher et al., Circulation (1998) 97: 645-650). Basic FGF enhances functional recovery and promotes neuronal sprouting following focal cerebral infarct (Kawamata et al., Proc. Natl. Acad. Sci. (1997) 94 (15):8179-84). According to the published literature, the FGF family consists of at least twenty-two members (Reuss et al., Cell Tissue Res. 313: 139-157 (2003)).

Fibroblast growth factor 21 (FGF-21) has been reported to be preferentially expressed in the liver (Nishimura et al., Biochimica et Biophysica Acta, 1492:203-206 (2000); WO 01/36640; and WO 01/18172, which are incorporated by reference herein) and described as a treatment for ischemic vascular disease, wound healing, and diseases associated with loss of pulmonary, bronchia or alvelor cells or function and numerous other disorders. FGF-21 is expressed primarily in liver, kidney, and muscle tissue (see Example 2 of US Patent Publication No. 20040259780 which is incorporated by reference herein in its entirety). The FGF-21 gene is composed of 3 exons and is located on chromosome 19. Unlike other FGFs, FGF-21 does not have proliferative and tumorigenic effects (Genome Biol. 2001; 2(3):REVIEWS3005).

US Patent Publication No. 20010012628, which is incorporated by reference in its entirety, describes a nucleotide and protein sequence for human FGF-21 (see SEQ ID NO: 1 and 2, respectively of US Patent Publication No. 20010012628). SEQ ID NO: 2 in the above-mentioned publication, referred to sbgFGF-19, is 209 amino acids in length and contains a 28 amino acid leader sequence at the N terminus. The human FGF-21 sequence presented as SEQ ID NO: 3 herein is the same sequence as SEQ ID NO: 2 of US Patent Publication No. 20010012628. This sequence has